REMARKS

Upon entry of this amendment, claims 1, 4, 5, 8–11, 14, 15, and 42 are pending in the application. Claims 2, 3, 6, 7, 12, 13, 16–41, and 43–49 are canceled. Claims 1 and 4 are amended.

Claim 4 is amended herein solely to change its dependency from claim 3 (now canceled) to claim 1. Support for the amendment to claim 1 can be found throughout the application, for example at paragraphs [0043], [0063], [0072]–[0073], and [0090] of the Application as published,[1] Examples 1 and 2, and original claims 3, 6, and 12. No new matter has been added by the present amendment. Applicants have amended the claims to expedite prosecution and reserve the right to pursue the original claims and any canceled claims in one or more continuing applications.

I. Rejection of Claims 1–3, 8–12, 14–15, 42–43, and 49 under 35 U.S.C. § 112, First Paragraph, as Failing to Comply with the Written Description Requirement

Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1–3, 8–12, 14–15, 42–43, and 49 under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

As an initial matter, Applicants note that claims 2, 3, 12, 43, and 49 have been canceled, and thus the rejection is moot with respect to those claims.

The Office states that the claims encompass a method of using any and all glutamate dehydrogenases (GDHs), isolated from any source, including any or all mutants, recombinants and variants thereof; and any or all variants of a GDH from *E. coli* comprising a leucine residue at position 92 and any other amino acids at any other position. The Office states that the specification fails to provide a sufficient written description of the claimed genus of proteins, because it "merely describes the functional

[1] All citations to Applicants' specification herein refer to the paragraphs as numbered in the specification as published as U.S. Patent Application Publication No. 2007/0009995.

features of the genus without providing any definition of the structural features of the species within the genus," and does not disclose which enzymes from the wide genus of GDHs are able to reduce the incorporation of norleucine into a polypeptide or which GDHs have norleucine degrading activity. The Office acknowledges that Applicants' specification describes a method of reducing the incorporation of norleucine into a heterologous polypeptide by co-expressing the heterologous polypeptide with either wild-type *E. coli* GDH or a mutant *E. coli* GDH having a lysine to leucine substitution at position 92 of the amino acid sequence.[2] However, Applicants understand the Office's position to be that these two examples do not constitute a sufficiently representative number of species to describe the entire claimed genus.

Without addressing the merits of the Office's reasoning, Applicants respectfully submit that claim 1 as currently amended complies with the written description requirement. Claim 1 has been amended herein to require that the non-standard amino acid degrading protein (NSAADP) consist essentially of a wild-type *E. coli* GDH or an *E. coli* GDH having a leucine at the amino acid position that corresponds to amino acid position 92 of the wild-type GDH, wherein the amino acid at position 92 of the wild-type GDH is a lysine. The specification provides sufficient written description support for claim 1 as presently amended, because the specification describes experiments showing that when either the wild-type *E. coli* GDH or the K92L variant of *E. coli* GDH were co-expressed with bovine somatotropin (bST), incorporation of norleucine into the bST was dramatically reduced (see Example 2 at paragraphs [0100]–[0101], and Table 5 in particular).

Applicants further submit that the use of the transitional phrase "consists essentially of" in the second clause of the body of claim 1 as currently amended is appropriate. The transitional phrase "consists essentially of" limits the scope of a claim to the specified materials "and those that do not *materially* affect the *basic* and *novel* characteristic(s)" of the claimed invention.[3] The skilled artisan would therefore

[2] Office Action dated May 26, 2011, at page 8.
[3] M.P.E.P. § 2111.03 (citing *In re* Herz, 537 F.2d 549, 551–52 (C.C.P.A. 1976) (emphases in original)).

understand that claim 1 as currently amended does not encompass "any and all variants of a glutamate dehydrogenase from *E. coli* comprising a leucine residue at position 92 and any other amino acids at any other position," as stated by the Office. Rather, the skilled artisan would understand that in addition to wild-type *E. coli* GDH and *E. coli* GDH having only the K92L variation, the claim encompasses only those GDHs having variations at amino acids other than lysine 92 which have norleucine degrading activity and which are effective in the claimed method of reducing incorporation of norleucine into a heterologous protein. As stated in Applicants' specification,

> Other aspects of this embodiment provide for recombinant DNA sequences encoding *E. coli* K92L GDH variants that further comprise variations at other amino acid residues. These variations are contemplated as being part of the instant invention *so long as they do not reduce the ability of the encoded protein to degrade norleucine to a degree that makes it unsuitable for use to prevent or substantially eliminate norleucine incorporation into a heterologous protein expressed in a cell.*[4]

Similarly, Applicants' specification further states:

> In other aspects of the present invention the GDH K92L variant may further comprise other variations from the native sequence. *All such variants are considered to be part of the instant invention so long as they do not diminish the protein's ability to degrade norleucine or other non-standard amino acids to a degree where it is no longer useful according to the instant invention.*[5]

The skilled artisan would readily be able to identify regions of the *E. coli* GDH which would likely be amenable to alteration without reducing the ability of the enzyme to degrade norleucine to a degree that makes it unsuitable for use to prevent or substantially eliminate norleucine incorporation into a heterologous protein. For example, the skilled artisan could use sequence alignment techniques which are well-known in the art to align the amino acid sequence of *E. coli* GDH with the amino acid

[4] Applicants' specification at paragraph [0090] (emphasis added).
[5] Applicants' specification at paragraph [0063] (emphasis added).

sequences of other GDHs of other bacterial species to identify conserved and non-conserved regions of the protein, and then make alterations within the non-conserved regions of *E. coli* GDH.[6] The skilled artisan would also readily be able to test such polypeptides for norleucine degrading activity and the ability to reduce or eliminate incorporation of norleucine into a heterologously expressed protein, for example by using the procedure set forth in Example 2 of Applicants' specification for testing the ability of a NSAADP to reduce or eliminate norleucine incorporation into bST.[7]

In view of the above, Applicants respectfully submit that claim 1 as amended herein complies with the written description requirement. Claims 8–11, 14, 15, and 42 each depend, directly or indirectly, from claim 1, and thus include all of the limitations of claim 1. Applicants therefore submit that claims 8–11, 14, 15, and 42 also comply with the written description requirement. Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1, 8–11, 14, 15, and 42 under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

II. Rejection of Claims 1–3, 8–12, 14–15, 42–43, and 49 under 35 U.S.C. § 112, First Paragraph, as Failing to Comply with the Enablement Requirement

Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1–3, 8–12, 14–15, 42–43, and 49 under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement.

As an initial matter, Applicants note that claims 2, 3, 12, 43, and 49 have been canceled, and thus the rejection is moot with respect to those claims.

The Office states that the specification is enabling for a method of reducing the incorporation of non-standard amino acids into a heterologous protein produced by a microorganism by transforming the microorganism with the heterologous polypeptide

[6] An example of such a sequence alignment is shown in Rice et al., which the Office has relied on in rejecting claims 3–5 and 12 as having been obvious under 35 U.S.C. § 103(a).
[7] As noted at paragraph [0100] of Applicants' specification, this assay methodology was previously described in a 1989 article by Bogosian et al.

and the glutamate dehydrogenase of SEQ ID NO. 2 or SEQ ID NO. 4. However, the Office states, the specification "does not reasonably provide enablement for a method of using any or all peptides having non-standard amino-acid degrading activity, but having unknown structure." The Office states that the claims encompass a method of using any and all GDHs, isolated from any source, including any or all mutants, recombinants and variants thereof; and any or all variants of a GDH from *E. coli* comprising a leucine residue at position 92 and any other amino acids at any other position. Therefore, the Office reasons, "the breadth of these claims is much larger than the scope enabled by the specification."

Without addressing the merits of the Office's reasoning, Applicants respectfully submit that claim 1 as currently amended is fully enabled by the specification. Claim 1 as amended herein requires that the NSAADP consist essentially of a wild-type *E. coli* GDH or an *E. coli* GDH having a leucine at the amino acid position that corresponds to amino acid position 92 of the wild-type GDH, wherein the amino acid at position 92 of the wild-type GDH is a lysine. Applicants' specification fully enables claim 1 as presently amended. For example, the specification describes experiments showing that when wild-type *E. coli* GDH or the K92L variant of *E. coli* GDH were co-expressed with bST, incorporation of norleucine into the bST was dramatically reduced (see Example 2 at paragraphs [0100]–[0101], and Table 5 in particular).

Furthermore, for the reasons stated herein in the section I above, Applicants submit that the use of the transitional phrase "consists essentially of" in the second clause of claim 1 as currently amended is appropriate. In particular, the skilled artisan would understand that claim 1 as currently amended does not encompass "any and all variants of a glutamate dehydrogenase from *E. coli* comprising a leucine residue at position 92 and any other amino acids at any other position," as stated by the Office. Rather, the skilled artisan would understand that in addition to wild-type *E. coli* GDH and *E. coli* GDH having only the K92L variation, the claim encompasses only those GDHs having variations at amino acids other than lysine 92 which have norleucine degrading activity and which are effective in the claimed method of reducing

incorporation of norleucine into a heterologous protein. As stated in Applicants' specification,

> Other aspects of this embodiment provide for recombinant DNA sequences encoding *E. coli* K92L GDH variants that further comprise variations at other amino acid residues. These variations are contemplated as being part of the instant invention *so long as they do not reduce the ability of the encoded protein to degrade norleucine to a degree that makes it unsuitable for use to prevent or substantially eliminate norleucine incorporation into a heterologous protein expressed in a cell.*[8]

The skilled artisan would readily be able to identify regions of the *E. coli* GDH which would likely be amenable to alteration without reducing the ability of the enzyme to degrade norleucine to a degree that makes it unsuitable for use to prevent or substantially eliminate norleucine incorporation into a heterologous protein. For example, the skilled artisan could use sequence alignment techniques which are well-known in the art to align the amino acid sequence of *E. coli* GDH with the amino acid sequences of other GDHs of other bacterial species to identify conserved and non-conserved regions of the protein, and then make alterations within the non-conserved regions of *E. coli* GDH. The skilled artisan would also readily be able to test such polypeptides for norleucine degrading activity and the ability to reduce or eliminate incorporation of norleucine into a heterologously expressed protein, for example by using the procedure set forth in Example 2 of Applicants' specification for testing the ability of a NSAADP to reduce or eliminate norleucine incorporation into bST. Although some amount of experimentation would be required to identify and test such *E. coli* GDHs having variations at amino acid residues other than lysine 92, Applicants submit that such experimentation would not be undue, but rather would be routine and well within the abilities of one having ordinary skill in the art. In particular, Applicants respectfully submit that the experimentation required would not be undue, since, in view of the current amendments to claim 1, any experimentation would be with respect to only a single GDH (i.e., an *E. coli* GDH).

[8] Applicants' specification at paragraph **[0090]** (emphasis added). *See also* paragraph [0063].

In view of the above, Applicants respectfully submit that claim 1 as amended herein is fully enabled by the specification. Claims 8–11, 14, 15, and 42 each depend, directly or indirectly, from claim 1, and thus include all of the limitations of claim 1. Applicants submit that claims 8–11, 14, 15, and 42 are each also fully enabled by the specification. Applicants therefore respectfully request reconsideration and withdrawal of the rejection of claims 1, 8–11, 14, 15, and 42 under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement.

III. Obviousness Rejections

A. Rejection of Claims 1–2, 8–11, 14–15, 42–43, and 49 under 35 U.S.C. § 103(a) as Having Been Obvious from Bogosian et al. in view of Wang et al.

Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1–2, 8–11, 14–15, 42–43, and 49 under 35 U.S.C. § 103(a) as having been obvious from Bogosian et al. in view of Wang et al.

As an initial matter, Applicants note that claims 2, 43, and 49 have been canceled, and thus the rejection is moot with respect to those claims.

The Office states that Bogosian et al. describes the incorporation of norleucine into bST when bST is expressed in *E. coli*, and discusses several methods for reducing the incorporation of norleucine into a target protein, including supplementing the media with methionine. The Office further states that Wang et al. describes a mutant GDH from *Clostridium symbiosum*, wherein the mutant GDH has a K89L mutation and increased activity for degrading norleucine. The Office reasons that one having ordinary skill in the art would have "recognized the advantage of expressing the mutant glutamate dehydrogenase of Wang et al. in order to directly degrade norleucine instead of indirectly reducing availability of norleucine." The Office further reasons that it would have been obvious from the cited references to reduce incorporation of norleucine into a heterologous protein by transforming *E. coli* with a vector (or two vectors) comprising

the heterologous protein and the mutant GDH of Wang et al. The Office asserts that since the mutant described by Wang et al. also degrades methionine, the skilled artisan would supplement the media with methionine to ensure enough availability of methionine. The Office further states that one having ordinary skill in the art would have been motivated to combine the Bogosian et al. and Wang et al. references in order to reduce incorporation of norleucine when producing heterologous proteins in *E. coli* and thereby produce heterologous proteins with minimal norleucine contamination. In addition, the Office states that the skilled artisan would have had a reasonable expectation of success, "since Wang et al. teaches a mutant enzyme which degrades norleucine and Bogosian et al. teaches expression of bovine somatotropin."

In response to Applicants' previous argument that the mutant GDHs of Wang et al. degrade the standard amino acid methionine in addition to norleucine, and thus such mutants would have been expected to adversely affect expression of heterologous proteins, the Office states: "In order to prevent degradation of methionine by the action of the mutant of Wang et al., one having ordinary skill would have recognized to add methionine to the media. This addition of methionine has two effects: ensures enough availability of methionine in production of the target protein and impeding norleucine from attaching to the $tRNA^{Met}$ and thereby reducing norleucine incorporation into the protein."

First, Applicants note that claim 1 as presently amended requires that the NSAADP consist essentially of a wild-type *E. coli* GDH or an *E. coli* GDH having a leucine at the amino acid position that corresponds to amino acid position 92 of the wild-type GDH, wherein the amino acid at position 92 of the wild-type GDH is a lysine. Each of claims 8–11, 14–15, and 42 depend, directly or indirectly, from claim 1, and therefore include all of the limitations of claim 1. Neither Bogosian et al. nor Wang et al. describes a wild-type *E. coli* GDH or an *E. coli* GDH having a leucine at the amino acid position that corresponds to amino acid position 92 of the wild-type GDH, wherein the amino acid at position 92 of the wild-type GDH is a lysine. Accordingly, even if Bogosian et al. and Wang et al. could be combined as proposed by the Office, one

skilled in the art would not arrive at the invention of claim 1. Thus, Applicants respectfully submit that for this reason alone, claims 1, 8–11, 14–15, and 42 would not have been obvious from the combination of Bogosian et al. and Wang et al.

In any event, Applicants respectfully disagree with the Office's contention that a skilled artisan would have been motivated to combine Bogosian et al. and Wang et al. The Office acknowledges that in addition to degrading norleucine, the mutant GDHs taught by Wang et al. also degrade the standard amino acid methionine. Moreover, the Office appears to agree with Applicants' argument that in view of this teaching of Wang et al., the skilled artisan would have expected the co-expression of the mutant GDHs of Wang et al. to negatively affect expression of heterologous proteins. Nevertheless, the Office maintains that a skilled artisan would have been motivated to combine the Bogosian et al. and Wang et al. references, and states that the skilled artisan would have simply supplemented the media with methionine in order to overcome the expected detrimental effect of the mutant GDH on heterologous protein expression.

However, as noted by the Office, Bogosian et al. recommends supplementation of the media with methionine in order to reduce the incorporation of norleucine into bST expressed in *E. coli*. Bogosian et al. explains that the rationale behind this approach is to prevent the charging of norleucine to $tRNA^{Met}$ by saturating the methioninyl-tRNA synthetase reaction with methionine.[9] Thus, Bogosian et al. describes the addition of methionine to the media as a solution to the problem of incorporation of norleucine into a heterologous protein expressed in *E. coli*. Bogosian et al. does not describe the co-expression of a GDH or any other NSAADP together with the bST. The Office has not explained why one skilled in the art would have been motivated to co-express a mutant GDH of Wang et al. in the bST-expressing *E. coli* of Bogosian et al. in order to reduce the incorporation of norleucine into the bST, when the skilled artisan would have expected in view of the teachings of Wang et al. that methionine supplementation would still be necessary. In other words, given that the skilled artisan would have expected that supplementation of the media with methionine would nevertheless be required,

[9] Bogosian et al., at p. 538, last full paragraph of left-hand column.

there would have been no reason to combine Bogosian et al. and Wang et al., and thereby add the additional step of co-expressing a GDH, when it was already known in the art that supplementation of the media with methionine could reduce incorporation of norleucine into a heterologous protein.

Applicants' specification explains that "It is desirable in the biotechnology industry to be able to cultivate recombinant organisms in a simple chemically defined minimal medium, without the need to add any expensive supplements, such as amino acids while simultaneously reducing the incorporation of norleucine into proteins," and that "Prior to the discovery of the invention disclosed in the instant application, there was no method known in the art that was able to achieve the objective of reducing the incorporation of norleucine into protein without requiring the supplementation of the culture medium with one or more amino acids and/or eliminating the methionine codons from the gene encoding the protein (thereby changing the protein's amino acid sequence)."[10] The specification emphasizes that "An important aspect of this invention is that it provides a means for achieving a reduction or elimination of the incorporation of norleucine and/or other non-standard amino acids into proteins without necessitating the supplementation of the culture medium with any amino acids."[11] A person having ordinary skill in the art would have been aware that supplementation of the media with amino acids such as methionine would be undesirable, and therefore would not have seen any reason to combine the Bogosian et al. and Wang et al. references, because he or she would have expected that in view of the methionine-degrading activity of the GDH mutants of Wang et al., supplementation of the media with methionine would still be required.

Although Applicants' specification acknowledges that in certain instances, amino acid supplementation may be desirable to even further reduce the incorporation of norleucine into a heterologous protein,[12] Applicants claimed invention achieves its

[10] Applicants' specification at paragraphs **[0024] & [0025]**. *See also* paragraphs [0025], [0030]–[0032], & [0043]–[0044].
[11] Applicants' specification at paragraph **[0032]**.
[12] *See* Applicants' specification at paragraph **[0045]**.

desired effect without any amino acid supplementation. As noted above, the method of Bogosian et al. requires supplementation of the media with methionine. To combine the disclosures of Bogosian et al. and Wang et al. would simply add the additional step of co-expressing a GDH. In sum, Applicants' claimed invention is directed to a manifestly different, simpler process that does not require amino acid supplementation, as compared to the process that would be provided by combining Bogosian et al. and Wang et al. One skilled in the art investigating such a process would have had no reason to combine the disclosures of Bogosian et al. and Wang et al.

The Office states that one having ordinary skill in the art would have "recognized the advantage of expressing the mutant glutamate dehydrogenase of Wang et al. in order to directly degrade norleucine instead of indirectly reducing availability of norleucine." However, it is unclear what particular "advantage" expressing the mutant GDH of Wang et al. would offer, when the skilled artisan would have expected that such expression would need to be accompanied by supplementation of the media with methionine, the solution to the problem of norleucine incorporation which had already set forth by Bogosian et al. Similarly, the Office states that the skilled artisan "would have been motivated to combine [Bogosian et al. and Wang et al.] in order to reduce incorporation of norleucine when producing heterologous proteins in *E. coli* and thereby produce heterologous proteins of interest with minimal norleucine incorporation," but does not explain what the source of this motivation would have been in view of the fact that the skilled artisan would have expected that it would continue to be necessary to supplement the media with methionine.

Moreover, Applicants respectfully submit that the skilled artisan also would not have had a reasonable expectation of success that combining Bogosian et al. and Wang et al. would lead to the identification of an alternative to the process of Bogosian et al. which requires amino acid supplementation. As explained above, the skilled artisan would have recognized that supplementation of the media with amino acids such as methionine was undesirable. Therefore, the skilled artisan would have been seeking an alternative means for reducing the incorporation of norleucine into heterologous

proteins which could avoid the need for such media supplementation. The skilled artisan would not have reasonably expected that combining Bogosian et al. and Wang et al. would provide such an alternative solution, because the skilled artisan would have expected in view of the methionine degrading activity of the GDH mutants of Wang et al. that supplementation of the media with methionine would still be required.

In view of the above, Applicants respectfully submit that the Office has not established a prima facie case of obviousness, since there would not have been any reason for a person having ordinary skill in the art to combine Bogosian et al. and Wang et al., and since the skilled artisan would not have had a reasonable expectation of success in combining these two references. Moreover, even assuming solely for the sake of argument that the Office had established a prima facie case of obviousness, Applicants' specification includes evidence of unexpected results sufficient to overcome any such prima facie case of obviousness. As explained above, in view of the teaching of Wang et al. that the GDH mutants described therein have methionine degrading activity, the skilled artisan would have expected that the co-expression of such GDH mutants together with bST would negatively affect expression of the bST, and that to overcome these negative effects, it would be necessary to supplement the media with methionine. Contrary to these expectations, Example 2 of Applicants' specification describes an experiment wherein bST was co-expressed with wild-type *E. coli* GDH or the K92L variant of the *E. coli* GDH (equivalent to the K89L mutant of *C. symbiosum* GDH described by Wang et al.). The transformed strains were grown on minimal medium, with no supplemental isoleucine, leucine, methionine, ALIMET®, rich medium supplement, or any other amino acid.[13] Despite this lack of media supplementation, co-expression with the *E. coli* K92L GDH variant nonetheless caused a dramatic reduction in the percent of bST containing norleucine. In particular, in the absence of co-expression of the *E. coli* K92L GDH variant, 17.4% of the bST contained norleucine, whereas when the bST was co-expressed with the K92L GDH variant, only 0.6% of the bST contained norleucine. This is a nearly 30-fold reduction. Such a result would not have been expected in view of the teachings of Wang et al.

[13] Applicants' specification at paragraph [0100].

In response to Applicants' unexpected results arguments made in the responses, the Office notes that the claims do not recite any limitation on the efficiency or yield of the expression of the heterologous protein, but simply require that the incorporation of norleucine is reduced. In addition, the Office states that the triple mutant of Wang et al. (i.e., the K98L/S380A/A163G mutant) only recognizes one natural amino acid (methionine), and recognizes two nonstandard amino acids (norleucine and norvaline). The Office again states that "Addition of methionine ensures enough availability of methionine in production of the target protein and impeding norleucine from attaching to the $tRNA^{Met}$ and thereby reducing norleucine incorporation into the protein."

With respect to the first of the Office's statements, Applicants respectfully submit that the fact that the claims do not quantify the reduction in the incorporation of norleucine into a heterologous protein does not preclude a finding of unexpected results. For the reasons stated above, the skilled artisan would not have expected that in the absence of any methionine supplementation, co-expression of the GDH mutants of Wang et al. with a heterologous protein such as bST would lead to reductions in incorporation of norleucine into the heterologous protein, let alone the dramatic reduction shown in Table 5 of Applicants' specification. Yet Applicants observed a nearly 30-fold reduction in the percent of bST which contained norleucine when the bST was co-expressed with the *E. coli* K92L GDH mutant. Applicants respectfully submit that such a dramatic reduction would not have been expected in view of the teachings of Wang et al. regarding the methionine-degrading activity of the mutants described therein.

With regards to the Office's comments regarding the triple mutant described in Wang et al., Applicants note that the Office has acknowledged that this mutant GDH has activity against methionine. In fact, as can be seen from Tables 2 and 4 of Wang et al., the activity of the triple mutant against methionine is substantial. In addition, at pH 7.0, the catalytic efficiency of the triple mutant towards L-methionine is comparable to its catalytic efficiency towards L-norleucine (see Table 4 of Wang et al.). Wang et al.

states: "In terms of natural amino-acid substrates, the triple mutant represents effective conversion of a glutamate dehydrogenase into a methionine dehydrogenase," and "The triple mutant shows a strong preference for L-Met and L-Nle as substrates. . . ."[14] Thus, it is clear from Wang et al. that the triple mutant has methionine-degrading activity, and a skilled artisan would have expected such activity to have an adverse effect on the expression of a heterologous protein such as bST. For the reasons explained above, in view of this teaching of Wang et al., a skilled artisan would not have had a reason to combine Bogosian et al. and Wang et al., and also would not have had a reasonable expectation of success in doing so to identify a method that does not require amino acid supplementation.

In view of the foregoing, Applicants respectfully submit that claims 1, 8–11, 14–15, and 42 would not have been obvious from the combination of Bogosian et al. and Wang et al., and therefore respectfully request reconsideration and withdrawal of the rejection of these claims under 35 U.S.C. § 103(a).

B. Rejection of Claims 3–5 and 12 under 35 U.S.C. § 103(a) as Having Been Obvious from Bogosian et al. in View of Wang et al. and Further in View of Rice et al.

Applicants respectfully request reconsideration and withdrawal of the rejection of claims 3–5 and 12 under 35 U.S.C. § 103(a) as having been obvious from Bogosian et al. in view of Wang et al. and further in view of Rice et al.

As an initial matter, Applicants note that claims 3 and 12 have been canceled, and thus the rejection is moot with respect to those claims.

For the reasons detailed below, Applicants respectfully submit that claim 1 (which incorporates the limitations of canceled claim 3) is not obvious in view of the cited combination of references.

[14] Wang et al., abstract, first full paragraph of right-hand column; page 5797, last paragraph in the right-hand column.

The Office states that it would have been obvious to reduce the incorporation of norleucine into bST in a microorganism by co-expressing in said microorganism the bST and a norleucine degrading mutant of Wang et al. The Office further notes that Wang et al. teaches that lysine 89, which corresponds to lysine 92 in the wild-type *E. coli* GDH, is in the substrate binding site. The Office also notes that the triple mutant taught by Wang et al. (K89L/S380A/A163G) no longer recognizes glutamate as a substrate, but does degrade methionine in addition to norleucine. The Office asserts that in view of this teaching, it would have been obvious to: (1) supplement the medium with methionine in order to ensure sufficient availability of methionine for the production of the target protein; or (2) make similar mutations (i.e., to make substitutions at the residues corresponding to residues 89, 380, and 163) in *E. coli* GDH, thereby obtaining a mutant having a greater substrate specificity towards norleucine. The Office further notes that Rice et al. discloses an alignment of three GDHs, including a GDH isolated from *E. coli*, with the GDH of *C. symbiosum*. The Office asserts that it would have been obvious in view of this alignment in Rice et al. for a skilled artisan to make mutations in *E. coli* GDH which correspond to the K89L, S380A, and A163G mutations in the *C. symbiosum* GDH triple mutant described by Wang et al. The Office states that the skilled artisan would have done this "in order to make an enzyme that has greater substrate specificity towards norleucine over other natural and non-standard amino acids and use said mutant enzyme to reduce incorporation of norleucine in heterologous proteins in *E. coli*." The Office asserts that the skilled artisan would have had a reasonable expectation of success because lysine 92 (*E. coli*)/lysine 89 (*C. symbiosum*) "is in the substrate binding pocket, and making site specific mutations are routine."

Applicants respectfully submit that for the reasons explained above in the preceding section, the skilled artisan would not have had a reason to combine Bogosian et al. and Wang et al., in view of the teaching of Wang et al. that the GDH mutants described therein have methionine-degrading activity. In addition, for the reasons explained in the preceding section, the skilled artisan also would not have had a reasonable expectation that co-expressing a mutant GDH of Wang et al. with a

heterologous protein, such as the bST of Bogosian et al., in *E. coli*, would yield an alternative means for reducing the incorporation of norleucine into the heterologous protein which did not require supplementation of the media with methionine. Moreover, as explained above, the data in Table 5 of Applicants' specification show that expression of *E. coli* K92L GDH unexpectedly reduced the percentage of bST containing norleucine by nearly 30-fold, in the absence of any amino acid supplementation. Moreover, with regards to the Office's remarks regarding the triple mutant, as explained above, the triple mutant of Wang et al. clearly exhibits methionine-degrading activity in addition to its norleucine-degrading activity. Therefore, even if the skilled artisan were to make the corresponding mutations in the *E. coli* GDH, the artisan would still have the expectation that this triple mutant GDH would degrade methionine and thereby adversely affect expression of a heterologous protein such as bST. The skilled artisan therefore would not have had a reason to combine the teachings of Bogosian et al. and Wang et al. to co-express such a triple mutant with a heterologous protein, and also would not have had a reasonable expectation of success.

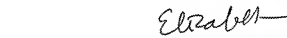
In view of the foregoing, Applicants respectfully submit that claims 4 and 5 would not have been obvious from the combination of Bogosian et al. and Wang et al., further in view of Rice et al., and therefore respectfully request reconsideration and withdrawal of the rejection of these claims under 35 U.S.C. § 103(a).

CONCLUSION

In view of the foregoing, Applicants respectfully request reconsideration and withdrawal of the rejections of claims 1, 4, 5, 8–11, 14–15, and 42.

The Commissioner is hereby authorized to charge any underpayment or credit any overpayment in connection with this Amendment to Deposit Account No. 19-1345.

Respectfully submitted,



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EEM/clp